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RESISTANCE GENES AND MARKERS FOR NET BLOTCH RESISTANT BARLEY FOR WESTERN CANADA

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Resistance genes and markers for net blotch resistant barley for western Canada

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(a) Abstract

The objectives were to identify net blotch resistance genes effective against *P. teres* virulences in western Canada, development of molecular markers and their use to transfer these genes into elite barley lines for durable resistance. Sources of resistance to net blotch were identified. Genetic studies in several populations revealed one to four resistance genes or quantitative trait loci (QTL). Screening of Australian barley populations with Canadian net blotch isolates demonstrated that molecular markers developed in one geographic region, may or may not be applicable in others. The major 6H QTL - *QRpt6* may provide NFNB adequate field resistance and could be routinely selected for using molecular markers in a practical breeding program for western Canada.

(b) Executive summary

Background

Net blotch of barley, caused by *Pyrenophora teres* Drechs. (anamorph: *Drechslera teres* (Sacc.) Shoemaker) is an important disease in western Canada (Tekauz 1990) and elsewhere (Steffenson 1997). Two types of leaf symptoms occur: the net form (NFNB), caused by *P. teres* f. *teres* and the spot form (SFNB), caused by *P. teres* f. *maculata* (Khan and Tekauz 1982). Yield losses of 20 to 30 % in susceptible cultivars have been reported in western Canada (van den Berg 1988) and up to 40 % in other parts of the world (Khan 1987). Thousand kernel weight is most often affected reducing quality as grain plumpness and malt extract are diminished.

Losses can be reduced by destruction of infected crop stubble, crop rotation, fungicide treatment and by growing resistant cultivars. Cultural and chemical control may not be feasible when using reduced tillage, wanting to minimize input costs and for organic production. Therefore, the most effective, economical control is via resistant cultivars.

Variation in *P. teres* virulence has been reported in most areas (Tekauz 1990, Steffenson and Webster 1992, Peever and Milgroom 1994, Gupta and Loughman 2001). Tekauz (1990) reported extensive pathogenic variability in western Canada and found that the most cultivars were susceptible to most isolates. Furthermore, the occurrence of virulent isolates on resistant accessions commonly used in resistance breeding programs emphasizes the need to identify and incorporate new resistance sources. Breeding for resistance should emphasize pyramiding of resistance genes for broad-based durable resistance.

Understanding the inheritance of resistance is valuable for planning crosses, identifying resistant genes, developing genetic markers to assist in selection and achieving efficient selection. Many major resistance genes and quantitative trait loci (QTLs) linked to net blotch resistance have been reported (Graner et al. 1996, Molnar et al. 2000, Steffenson et al. 1996, Richter et al. 1998, Raman et al. 2003, Williams et al. 2003, Gupta et al. 2004). The number and location of resistance genes is dependent on plant genotype, plant growth stage, pathogen isolate and plant growth environment. In general, a high

correlation between seedling resistance and adult-plant resistance has been observed (Tekauz 1986, Gupta and Loughman 2001) indicating that screening at the seedling stage can be effective in selecting for resistance. However, traditional phenotypic screening for seedling resistance is costly and only limited number of breeding lines can be reasonably screened in this fashion.

Testing western Canadian cultivars with local *P. teres* isolates showed that CDC McGwire was the best available cultivar (Tekauz 2000). There is a need to transfer this resistance efficiently into other elite breeding lines. High variability in the pathogen indicates the need to identify novel resistance genes and combine them efficiently with the available resistance.

Molecular markers linked to resistance genes allow rapid introgression of resistant genes into elite lines and the possibility to pyramid resistance genes in new selections. In addition, markers can be used in the absence of pathogens, lead to a better understanding of the disease and facilitate the isolation of new resistance genes.

Molecular markers linked to net blotch resistance have been reported from Australia (Raman et al. 2003, Cakir et al. 2003, Williams et al. 2003, Gupta et al. 2004). However, *P. teres* virulences in Canada are different from Australia (Gupta et al. 2003); for example while virulences in western Australia have not changed over a 20-year period, in western Canada the pathogen is highly variable (Tekauz 1990). Validation of the Australian markers in Canadian populations and identification of resistance genes effective against *P. teres* virulence in western Canada was needed to utilize and develop markers for these genes. Molecular markers can accelerate the transfer of resistant genes into elite lines and will help pyramid multiple resistance genes into new populations. Cultivars with greater resistance to net blotch will sustain and improve the yield, quality and marketability of Canadian barley.

Objectives

1. Evaluate the variability of resistance in local and introduced barley genotypes against local *P. teres* isolates
2. Study the inheritance of resistance in resistant barley lines/cultivars
3. Identify and evaluate molecular markers linked to net blotch resistance
4. Transfer various sources of net blotch resistance into high yielding lines using MMAS and combine those with effective resistance to other important pathogens.

Results

Screening 42 barley lines with six NFNB (WRS102, WRS858, WRS1607, WRS1906, LO256, LO246) and four SFNB (WRS857, WRS1881, LO233, LO231) isolates at the seedling stage in the University of Saskatchewan Phytotron identified three lines (TR253, Kao 32-12 and CI9214) resistant to all isolates. Overall, CI9214, a 6-row Korean line, was the most resistant and the European 2-row variety Alexis was the most susceptible.

There was a significant line × isolate interaction ($P < 0.0001$) indicating specificity in the host-pathogen relationship. NFNB isolates were more virulent than SFNB isolates.

No line showed adult-plant susceptibility combined with seedling resistance, however, a few lines were more resistant as adult plants. This was particularly notable for NFNB isolate WRS858. CDC Bold, Chebec, Stirling, Kaputar, Baudin, Hamelin showed resistance to WRS858 at the flag leaf in contrast to susceptibility at the seedling stage suggesting the presence of adult-plant resistance genes in these lines. Field testing of 42 selected lines at Melfort, SK in 2005, 2006 and 2007 for NFNB showed a strong correlation ($r=0.73$-0.81) between adult-plant disease reaction in the field and seedling disease reaction (average of 6 *P. teres* f. *teres* isolates) in the Phytotron. The strong agreement between seedling reaction and adult-plant reaction indicated that seedling screening could be useful to select for field resistance. Screening at the seedling stage in growth cabinets has several advantages over field screening: many populations can be screened in a short time; confounding effects of other diseases can be avoided; continuous screening throughout the year; individual isolates can be used; optimum humidity, temperature and light conditions conducive to clear symptoms expressions can be maintained and repeated over experiments. However, compared with MMAS (molecular marker assisted selection), number of lines screened will still be limited.

Several doubled-haploid (DH) and Recombinant Inbred Line (RIL) barley populations were screened at the seedling stage in the Phytotron and/or at the adult-plant stage in the field at Melfort, SK to study the inheritance of resistance. Chi-square tests indicated segregation for one to four resistance genes depending upon isolate, resistant source, plant growth stage and plant growth environment used. High heritability for different tests revealed that the majority of the phenotypic variance was due to genetic effects.

To evaluate whether we could use Australian identified molecular markers under western Canadian situations, the parents of Australian barley mapping populations used to identify/validate molecular markers were screened against Canadian net blotch isolates. Franklin, Kaputar, Keel, Baudin and Hamelin (resistant to NFNB in Australia) were susceptible to the majority of the Canadian NFNB isolates tested indicating resistance markers identified in Australia would not be useful for Canadian breeding programs. However, lines WA4794, Pompadour, Halcyon, Tilga and Chebec showed a resistant reaction to some isolates. Interestingly, CI9214, CI5791, TR251, Heartland, WPG8412 and WA4794 showed resistance to many Australian (Gupta et al. 2003, Gupta et al. 2006) as well as Canadian NFNB isolates (in this study) suggesting that genes present in these lines may be effective against a wide range of isolates. Similarly, CI9214, TR251, Tilga and Keel showed resistance to many Australian (Williams et al. 2003, Gupta et al. 2006) as well as Canadian SFNB isolates (in this study).

Symptoms on the Australian cultivar Gairdner infected by many NFNB isolates were atypical, being more similar to SFNB symptoms. PCR analysis of ten *P. teres* isolates with form-specific primers produced a 378 bp band in *P. teres* f. *teres* (NFNB) isolates and a 411 bp band in *P. teres* f. *maculata* (SFNB) isolates confirming their identity. The

atypical resistance in Gairdner to many NFNB isolates may be due its specific host reaction which needs to be investigated.

Evaluation of inheritance of resistance in four Australian populations against western Canadian *P. teres* isolates revealed one to three resistance genes depending upon isolate used. High heritability (0.86-0.95) for inheritance revealed that phenotypic variance is mainly due to genetic effects. The Pompadour/Stirling population showed one and three gene segregation to NFNB isolates WRS1607 and WRS102, respectively. Cakir et al. (2003) and Gupta et al. (2004) reported single gene segregation in this population to several NFNB isolates. A strong correlation between disease reaction of WRS1607 and the Australian isolate 95B100 indicates that the same 6H gene/QTL was effective against isolate WRS1607.

The CI9214/Stirling population segregated for three resistance genes for NFNB isolate WRS858. Gupta et al. (2004) also reported three gene segregation for resistance to Australian NFNB isolates NB50 and NB52B and mapped four genes on 6H, 4H, 3H and 2H depending on pathotype and plant development stage. QTL analysis identified two regions on chromosome 3H with a maximum R^2 value of 9% associated with seedling resistance to WRS858. Similarly, Gupta et al. (2004) identified a major and minor gene on 3H associated with seedling resistance to NB50 and NB52B, respectively showing the same gene(s) is effective against some western Australian and a western Canadian isolate indicating the markers linked to this gene identified in Australia may be useful for MMAS in Canada. This population showed single gene resistance for seedling SFNB resistance to isolate WRS857. Similarly, Williams et al. (2003) reported single gene (*Rpt4*) resistance to SFNB at the seedling stage in this population. However, the seedling stage reaction of this population to WRS857 and Australian isolates was not correlated (r = -0.05) indicating that *Rpt4* is not effective against WRS857. This was further confirmed by QTL analysis of this population identifying a major QTL on 6H and minor QTL on 4H and 2H to isolate WRS857.

Seedling resistance in Chebec/Harrington to SFNB isolate LO233 was controlled by a single gene. This was further confirmed by mapping a single major seedling resistance QTL to chromosome 7H near to PSR167b, at the same position as *Rpt4* (Williams et al. 2003). Markers linked to *Rpt4* could be used to select lines carrying this gene. However, *Rpt4* is of little practical value for the western Canadian barley breeding programs as it is not effective against SFNB isolate WRS857, the long-term standard Canadian SFNB isolate used to screen elite barley lines for SFNB resistance reaction.

Segregation analysis in the Halcyon/Sloop population against NFNB isolate WRS858 revealed that seedling resistance is controlled by more than one gene. Raman et al. (2003) reported a major seedling resistance QTL on 4H (*QRpts4L*) in this population to Australian NFNB isolate NB50. The strong correlation (r= 0.70) between infection response for these two isolates indicates the same resistance QTL is effective against WRS858.

In conclusion, this study reveals that molecular markers developed in one geographic region, may or may not be applicable in others due to the differences in germplasm and fungal pathogen strains used in different regions or countries. The majority of Australian mapping barley population parents (resistant to NFNB in Australia) were susceptible to Canadian net blotch isolates suggesting markers linked to their resistance will not be useful for the Canadian barley breeding programs; however, several net blotch resistance genes or QTL (*QRpts6*, *QRpts4*, 3H genes for NFNB and *Rpt4* for SFNB) were effective against both Australian and Canadian isolates indicating that molecular markers linked to these genes or QTL could be used for MMAS in Canadian barley breeding programs. It is relatively faster and less expensive to validate identified molecular markers than to develop new ones. If applicable, these could be used for MMAS in local breeding programs. We found that marker HVM74 linked to *QRpt6* is useful for CDC barley breeding program. However, it is also important to identify novel resistance genes to local pathogen populations and develop molecular markers linked to these genes to be used in introgressing/pyramiding these genes into elite lines.

QTL associated with net blotch resistance were identified in the DH population CDC Dolly/TR251 using Diversity Arrays Technology (DArT®) markers. One hundred and fifty DH lines were screened as seedlings in controlled environments with NFNB isolates WRS858 and WRS1607 and SFNB isolate WRS857. The population was also screened at the adult-plant stage for NFNB resistance in the field in 2005 and 2006. A high-density genetic linkage map of 90 DH lines was constructed using 557 DArT® and 11 SSR markers. A major NFNB seedling resistance QTL, designated *QRpt6*, mapped to chromosome 6H for isolates WRS858 and WRS1607. *QRpt6* was associated with adult-plant resistance in 2005 and 2006 field trials. Additional QTL for NFNB seedling resistance to the more virulent isolate WRS858 were identified on chromosomes 2H, 4H, and 5H. A seedling resistance QTL (*QRpts4*) for the SFNB isolate WRS857 was detected on chromosome 4H as was a significant QTL (*QRpt7*) on chromosome 7H. Three QTL (*QRpt6*, *QRpts4*, *QRpt7*) were associated with resistance to both net blotch forms and lines with one or more of these demonstrated improved resistance. Simple Sequence repeat (SSR) markers tightly linked to *QRpt6* and *QRpts4* were identified and validated in an unrelated barley population, MEH#486/Harrington. QTL analysis of the CDC Bold/TR251 population also demonstrated the 6H QTL was associated with NFNB seedling and adult-plant resistance. Interestingly, some of the QTL identified in CDC Bold/TR251 population were different from that identified in the CDC Dolly/TR251 population suggesting a background effect of the susceptible parent on QTL expression. The major seedling and adult plant resistance QTL/gene on 6H in TR251 and MEH#486 may be sufficient to provide adequate field resistance to NFNB and could be selected for based on Phytotron screening or using molecular markers.

Screening the parents of 22 barley populations with SSR markers HVM74 and HVM03 linked to *QRpt6* and *QRpts4*, respectively showed that these SSR markers could be used for practical MMAS in many barley populations available in the CDC barley breeding program. Four barley populations were screened with SSR markers HVM74 and HVM03 to select lines resistant to both forms of net blotch. The selected lines were screened at the seedling stage with NFNB isolate WRS858 and SFNB isolate WRS857 and at the adult-

plant stage in the field at Melfort. The results demonstrated that MMAS resistant lines had significantly lower infection response than susceptible lines, indicating that MMAS for net blotch resistance is practical. Since the major 6H QTL, *QRpt6*, may provide adequate NFNB field resistance in western Canada, the practical application plan for the Crop Development Centre barley breeding program is to routinely utilize MMAS for *QRpt6* followed by normal field screening in field net blotch nurseries to identify additional resistance provided by the other QTL. Where feasible and resource permitting the CDC program has implemented routine MMAS for *QRpt6* and will recommend this to other breeding programs.

(c) Technical Report

1. Evaluate variability in local and introduced barley genotypes against *P. teres* isolates

1.1 *Pyrenophora teres* isolates

Six *P. teres* f. *teres* isolates (WRS102, WRS858, WRS1906, WRS1607, LO246, LO256) causing net-form net blotch (NFNB) and four *P. teres* f. *maculata* isolates (WRS857, WRS1881, LO231, LO233) causing spot-form net blotch (SFNB) were used to screen barley lines at the seedling stage in the University of Saskatchewan Phytotron (Table 1).

Table 1. Isolates of *Pyrenophora teres* used in this study.

Isolate	Location Collected	Year collected	Reference
[†]NFNB			
[‡]WRS102	SK	1959	McDonald (1967)
WRS858	Teulon, MB	1973	Tekauz and Mills (1974)
WRS1607	Prince Albert, SK	1985	Tekauz (1990)
WRS1906	NB	1994	A. Tekauz (Pers. comm.)
[§]LO246	Nevis, AB	2004	K. Turkington (Pers. Comm.)
LO256	Blackfalds, AB	2004	K. Turkington (Pers. Comm.)
SFNB			
WRS857	Oakbank, MB	1973	Tekauz and Mills (1974)
WRS1881	Saskatoon, SK	1993	A. Tekauz (Pers. comm.)
LO231	Delburne, AB	2004	K. Turkington (Pers. Comm.)
LO233	Blindman valley, AB	2004	K. Turkington (Pers. Comm.)

[†]NFNB- net-form net blotch (*P. teres* f. *teres*); SFNB- spot-form net blotch (*P. teres* f. *maculata*)

[‡]WRS isolates were obtained from E. Mueller and A. Tekauz, AAFC, Cereal Research Centre, Winnipeg, MB.

[§]LO isolates were obtained from D. Orr and K. Turkington, AAFC, Lacombe, AB.

1.2 Inoculum production

Isolates were sub-cultured on V8 Juice Agar (V8A) plates and incubated at 21±1°C with a 12-h photoperiod. After 10-14 days, conidia were harvested by adding 10 ml of sterile distilled water to the petri plate, scrapping the culture with a glass rod and filtering through two layers of cheesecloth. Conidia per milliliter were counted with a hemacytometer and the concentration was adjusted to 10^4 conidia/mL.

1.3 Screening of barley genotypes at the seedling stage

Forty-two barley lines were selected for screening at the seedling stage with 10 *P. teres* isolates. These included resistant and susceptible lines from western Canadian barley breeding programs (including parents of potential segregating populations); parents of Australian barley mapping populations used to identify/validate net blotch resistance molecular markers in Australia; USA lines; two Ethiopian accessions and other lines from International collections. All lines were 2-row type except Heartland and CI9214. All except Halcyon were spring type. Seeds were surface sterilized in 0.12% formalin for 1 hr and then rinsed in running water for 10 min and dried over-night.

1.4 Inoculation of plants

Eight seeds/line were sown in a bunch with three lines per 15 cm pot containing Sunrise® LG3 Mix and raised in a growth chamber at 24/18°C with 16/8 h photoperiod. Three replications of each line were planted in a randomized complete block design. Plants were inoculated 14 d after seeding (three leaf stage) with 10,000 spores/ml (10-14 d old fungal culture) using a Duray® 2 oz. Pump Sprayer (Goody Products, Inc., Peachtree City, GA). Sufficient inoculum was applied until the plants were uniformly wet, approximately 5 mL per pot. Inoculated plants were incubated at 21°C in darkness for 24 h at 100% RH. Inoculated plants were then grown at 21/19°C with 16/8 h photoperiod at 75% RH.

1.5 Assessment of infection response

Scoring was performed 8 d after inoculation on a 1-10 scale for *P. teres* f. *teres* (net form) and on the same scale but without employing categories 4, 6 and 10 for *P. teres* f. *maculata* (spot form) (Tekauz 1985). Generally the most common lesion type was scored. Lines were classified as resistant (1.0-3.0), intermediate (3.1-5.0) and susceptible (>5.0) based on infection response (IR). For each isolate, the test was conducted once and repeated only if symptoms were not fully expressed on susceptible checks.

1.6 Statistical analysis

Statistical analysis was performed using SAS (SAS Institute Inc. 2005). The infection response data from NFNB and SFNB isolates were pooled separately and an analysis of variance (ANOVA) was calculated. The ANOVA was also calculated in a combined analysis over all 10 isolates.

1.7 Results and Discussion

The 42 lines tested showed variable infection response to *P. teres* isolates ranging from 1 to 9. Disease development progressed rapidly and symptoms appeared on susceptible cultivars within 48 h after inoculation. NFNB isolates were more virulent than SFNB isolates. Isolate LO256 (from Blackfalds, Alberta) was the most virulent followed by isolate WRS858. SFNB isolate WRS1881 was the least virulent. There was a significant line × isolate interaction ($P < 0.0001$) indicating host-pathogen specificity. Nine lines were classified resistant to NFNB isolates whereas 12 lines showed resistance to SFNB isolates. Three lines TR253, Kao 32-12 and CI9214 were resistant to all NFNB and SFNB isolates. Of note, symptoms on the Australian variety Gairdner to many NFNB isolates were not typical net form but were elliptical spots similar to spot form symptoms. Moderate resistance in Gairdner to many NFNB isolates was unique. Gairdner was susceptible to all SFNB isolates. Overall CI9214, a 6-row South Korean line, was the most resistant and the European variety Alexis was the most susceptible to all NFNB and SFNB isolates. CI5791, an Ethiopian 2-rowed line, was resistant to all NFNB isolates tested as has been reported in many other studies from different countries (Buchannon and McDonald 1965 - Canada, Steffenson and Webster 1992 - USA, Alfanasenko et al. 1995 -Germany, Russia, Czech Republic, Slovakia, Khan 1982 and Gupta et al. 2003 - Australia). However, CI5791 showed an intermediate reaction to many SFNB isolates. This observation is in agreement with that of Tekauz and Mills (1974). On the other hand, Manley, Tilga and Keel were resistant to many SFNB isolates but susceptible to NFNB isolates. Tekauz (1990) reported the increased prevalence of *P. teres* f. *maculata* in Saskatchewan and Alberta. The sources of resistance to net and spot form of *P. teres*, used in breeding programs are mainly derived from CI5791 and CI9214, respectively. There is need to combine resistance to both forms of net blotch in elite breeding lines. The selected 42 barley lines were screened at the adult-plant stage with isolates WRS858 and WRS857 in the Phytotron. There was a strong correlation between seedling reaction and adult-plant stage reaction for WRS858 ($r=0.71$) and WRS857 ($r=0.80$). No line was observed that showed adult plant susceptibility combined with seedling resistance.

1.8 Field screening of selected barley lines

The 42 lines were evaluated in the 2005, 2006 & 2007 CDC NFNB field disease screening nursery at Melfort, SK where NFNB is endemic. Natural infection occurred (no artificial inoculum was applied). Spreader rows of the highly susceptible cultivar Harrington were grown around the experiment for pathogen development and spread. In the field, lines were planted as hill plots (10-15 seeds/hill) with experiments arranged in a randomized complete block design with three replications per line. To control weeds, a tank mix of the herbicides Prestige /Achieve liquid gold was applied at the recommended rate. NFNB reaction was scored using a 1-9 scale where 1 is highly resistant and 9 is highly susceptible.

In field trials, typical symptoms of NFNB occurred on the upper leaves of susceptible lines. High disease levels on the susceptible cultivar Harrington in all years

confirmed epidemic severity. Harrington was most susceptible and C283612 and CI9214 most resistant. There was a strong correlation (r=0.74 to 0.83) between disease reaction at the adult-plant stage in the field and Phytotron seedling disease reaction (average of 6 *P. teres* f. *teres* isolates). No seedling resistant line showed adult-plant susceptibility in the field, however, many lines viz. CDC McGwire, CDC Helgason, Halcyon and WPG8412 showed field resistance but only a moderately resistant seedling reaction. 2006 and 2007 disease severity was less than 2005, however, the disease reaction correlation among lines across the years was strong (r=0.73-0.81). The strong agreement between seedling and adult-plant reaction indicates that seedling screening could be used to select for field resistance. Screening at the seedling stage in growth cabinets has several advantages over field screening: many populations can be screened in a short time; confounding effects of other diseases can be avoided; continuous screening throughout the year; individual isolates can be used; optimum humidity, temperature and light conditions conducive to clear symptoms expressions can be maintained and repeated over experiments. However, MMAS would be more efficient.

1.9 PCR assays of *P. teres* isolates

As noted in section 1.7, symptoms on the Australian cultivar Gairdner infected by many NFNB isolates were atypical, and similar to SFNB symptoms. Net blotch lesions on barley leaves caused by net and spot forms can be confused with each other (particularly in the field) making it difficult to correctly identify isolates from plant samples. Both pathogens produce similar cultures on media and conidia are very similar in shape and morphology. Australian researchers, Williams et al. (2001) developed a PCR assay to identify different forms of *Pyrenophora* directly from infected plant tissues. We used this PCR assay to confirm the identity of Canadian isolates used in this study (to our knowledge this was not performed earlier in Canada).

Fungal mycelia of ten *P. teres* isolates were used for DNA extraction. Fungal conidia harvested from 10-14 day old V8A culture plates were grown in potato dextrose broth (PDB) for 48 h in a shaker (150 rpm) at room temperature. Mycelium was harvested from PDB and freeze dried for 72 h at –50°C in a Heto Lyolab 3000 freeze-dryer (Heto-Holten A/S, Allerod, Denmark). DNA was extracted from fungal mycelia using the CTAB method (Procunier et al. 1991). PCR primers specific to *P. teres* forms (PTT – *P. teres* f. *teres*; PTM – *P. teres* f. *maculata*), developed by Williams et al. (2001), were used in a single multiplex reaction. Amplification was performed in a total volume of 25 µL, containing 100 µM each of dNTPs (Gibco BRL), 200 nM of each primer, 10 ng of fungal DNA, 2.0 mM of $MgCl_2$ (Gibco BRL), 1 unit of *Taq* and 1X of Gibco BRL buffer (50 mM KCL, 20 mM Tris-HCL, pH 8.4). Amplification was performed with a GeneAmp® PCR system 9600 (Perkin Elmer, USA) using a touchdown cycling protocol which consisted of an initial denaturation step at 94°C for 3 min, followed by 35 cycles as follows: a denaturation step at 94°C for 30 s, an annealing step at 65°C for 30 s reducing 1°C per cycle to 56°C and an extension step at 72°C for 30 s; ending with an extension period of 72°C for 10 min. Amplified products were separated on 1.5% agarose, 0.5 X TBE gel, stained with ethidium bromide and viewed under ultra-violet light.

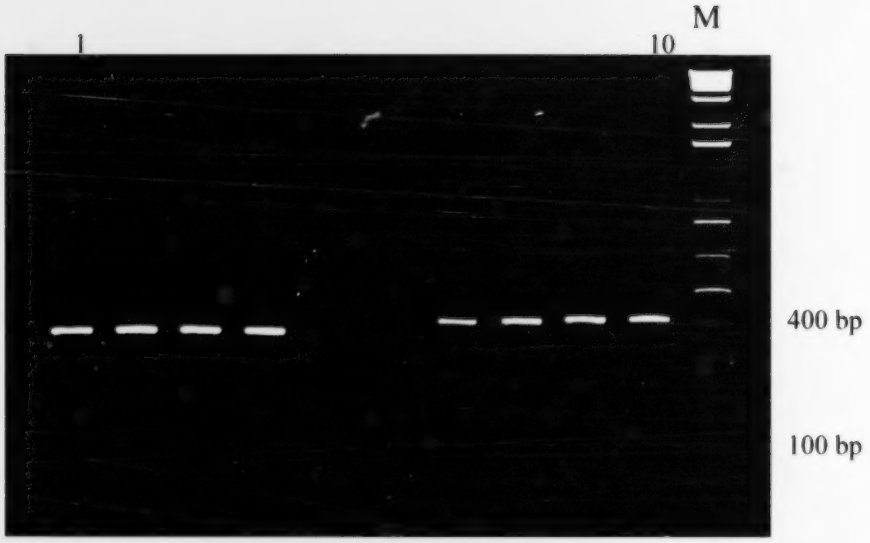

Fig. 1. Amplification products obtained with PTT and PTM primers from a single multiplex PCR reaction. Lanes 1-6: *P. teres* f. *teres* isolates WRS102, WRS858, WRS1607, WRS1906, LO256, LO246 showing 378 bp band; Lanes 7-10: *P. teres* f. *maculata* isolates WRS1881, WRS857, LO231, LO233 showing 411 bp band. M is 1 kb+ ladder.

PCR analysis of ten *P. teres* isolates with form-specific primers produced a 378 bp band in *P. teres* f. *teres* (NFNB) isolates and a 411 bp band in *P. teres* f. *maculata* (SFNB) isolates (Fig. 1) confirming their identity. The atypical resistance in Gairdner to many NFNB isolates may be due its specific host reaction which needs to be investigated.

1.10 Evaluation of international germplasm to identify potential new sources of resistance

We have barley lines with *H. bulbosum* chromosome substitution(s) obtained from Dr. Richard Pickering, New Zealand Institute for Crop & Food Research Limited, Christchurch, New Zealand. Some of these lines showed resistance to powdery mildew (Pickering 1992, Pickering et al. 1995), scald (Pickering 1992, Singh et al. 2003) and other diseases (Pickering et al. 1987). The purpose of this study was to determine their reaction to Canadian net blotch isolates.

Fifty New Zealand lines were evaluated against NFNB isolate WRS858 and SFNB isolate WRS857 at the seedling stage in the Phytotron as previously described. All lines were susceptible or highly susceptible to WRS858 indicating no resistance to NFNB in this material. For the SFNB isolate, four lines showed intermediate resistance and all others were susceptible.

2. Inheritance of resistance in resistant barley lines

Several doubled-haploid (DH) and Recombinant Inbred Line (RIL) populations were screened at the seedling stage in the Phytotron and/or at the adult-plant stage in the field at Melfort, SK to study inheritance of resistance.

2.1 CDC Dolly/TR251 population

One hundred and fifty DH lines from the CDC Dolly/TR251 population were screened at the seedling stage with NFNB isolates WRS858 and WRS1607 and SFNB isolate WRS857. There were three replications of each line for each isolate. The planting, inoculum production, inoculation and scoring was as described in section 1. There were three sub-experiments for each isolate. Three replications of each DH line were planted using an incomplete block design with a random sub-set of DH lines assigned to each sub-block along-with parents and checks (Harrington and TR253). Due to limitations in screening area, each incomplete block was separated in time. The 150 lines were also screened at the adult-plant stage in the field in NFNB disease screening nursery at Melfort, SK in 2005, 2006 and 2007 with three completely randomized replications for each line. Other conditions were as described in section 1.9.

Analysis of variance was performed using PROC Mixed of SAS 9.1 (SAS Institute Inc. 2005). Parental lines, checks, and DH lines were considered fixed effects. Least significant differences (LSD) were calculated ($P = 0.05$) for each test for mean comparisons. Heritability was calculated for each test using variance estimates derived from PROC Mixed. The phenotypic variance was $\sigma^2_{\text{DH lines}} + \sigma^2_{\text{residual}}/\text{replications}$. For heritability analysis, all factors were considered random.

TR253 and Harrington were used as resistant and susceptible checks, respectively. TR251 was consistently resistant in all the tests and CDC Dolly was susceptible (Table 2) except in the field in 2006 and 2007 where CDC Dolly showed an intermediate reaction. The response of resistant and susceptible checks was as expected. NFNB isolate WRS858 was more virulent than isolate WRS1607 at the seedling stage, as evidenced by the significantly higher IR (T-test, P =0.05) for CDC Dolly and TR251. High heritability for different tests (0.72-0.91) revealed that the majority of the phenotypic variance was due to genetic causes.

Chi-square tests indicated segregation for one to four resistance genes against NFNB isolates and natural infection in the field (Table 3) except 2007 field experiment where all the lines rated below 5. Many lines showed intermediate reactions indicating the presence of minor genes/modifiers contributing NFNB resistance. This population segregated for three resistance genes against SFNB isolate WRS857.

Table 2. Net blotch infection response (1-10) of parents and checks of the CDC Dolly/TR251 population

Line/Test	[a]NFNB				SFNB
	[b]WRS858	[b]WRS 1607	Field 2005	Field 2006	Field 2007	[b]WRS857
TR251	2.8	2.0	1.8	1.4	1.7	2.3
CDC Dolly	8.1	6.3	6.5	4.3	3.8	7.6
TR253	1.2	1.7	2.2	1.6	2.0	2.9
Harrington	8.2	7.9	8.7	7.8	8.5	8.9
Popul- Mean	5.2	3.5	4.0	2.7	2.5	6.4
ation Range	2.0-8.3	1.7-7.3	2.0-6.7	1.3-6.0	1.0-4.3	2.7-8.0
[c]$LSD_{0.05}$ lines	1.4	1.1	1.4	1.4	1.2	1.3
$LSD_{0.05}$ lines vs. checks	1.2	0.9	1.4	1.4	1.2	1.1
Heritability	0.90	0.91	0.84	0.72	0.75	0.82

[a]NFNB - Net form net blotch. SFNB - Spot form net blotch
[b]*Pyrenophora teres* isolate.
[c]Least significant differences (P=0.05)

Table 3. Chi-square analysis of CDC Dolly/TR251 population reaction to net blotch

	Isolate/Test	# of R lines	# of S lines	R:S ratio	χ^2 Value	P value	# of genes*
NFNB	WRS858	83	67	1:1	1.17	0.19	one
	WRS1607	133	17	7:1	0.19	0.66	three
	Field 2005	123	27	3:1	3.92	0.05	two
	Field 2006	148	2	15:1	6.22	0.01	four
	Field 2007	150	0	No segregation			
SFNB	WRS857	23	127	1:7	1.07	0.3	three

*Other gene ratios were evaluated and only those with the best fit are given here.

2.2 CDC Bold/TR251

One hundred and fifty DH lines were screened at the seedling stage with NFNB isolates WRS858 and WRS1607 and SFNB isolates WRS857 and LO233 in the Phytotron. The population was also screened at the adult-plant stage in the field in 2006, 2007 and 2008 at the CDC NFNB nursery at Melfort, SK. This population was selected to compare the resistance of TR251 in different backgrounds (CDC Dolly and CDC Bold). CDC Bold was moderately susceptible to many NFNB isolates whereas CDC Dolly was susceptible to all six isolates at the seedling stage. Materials & methods and data analysis as described in section 2.1.

CDC Bold was susceptible at the seedling stage to NFNB isolate WRS858, SFNB isolates WRS857 & LO233 and showed intermediate reaction to NFNB isolate WRS1607 (Table 4). CDC Bold showed a susceptible reaction at the adult-plant stage in the field in

2006, 2007 and 2008. Interestingly, CDC Bold showed less disease at the seedling stage but more disease at the adult-plant stage than CDC Dolly. TR251 was consistently resistant in all the tests. The response of resistant and susceptible checks was as expected. High heritability for different tests (0.74-0.93) revealed that the majority of the phenotypic variance was due to genetic effects.

Table 4. Infection response of parental lines and checks to net blotch in CDC Bold/TR251 population

Line/Test		[a]NFNB				SFNB	
	[b]WRS858	[b]WRS 1607	Field 2006	Field 2007	Field 2008	[b]WRS857	LO233
TR251	3.0	1.8	1.4	2.0	2.1	1.8	4.8
CDC Bold	6.7	4.6	5.8	6.3	5.4	5.4	7.2
TR253	1.4	1.4	1.6	2.0	1.9	2.4	3.7
Harrington	8.7	6.7	7.8	8.5	7.8	7.8	6.9
Popul Mean	5.7	4.0	2.9	3.5	3.5	4.2	6.6
ation Range	2.0-9.3	1.0-7.7	1.0-8.0	1.3-7.7	1.7-7.3	1.0-7.3	3.7-8.0
[c]$LSD_{0.05}$ lines	1.5	1.5	[d]NA	1.4	1.3	1.4	1.4
$LSD_{0.05}$ lines vs. checks	1.2	1.2		1.4	1.3	1.1	1.2
Heritability	0.93	0.92		0.89	0.90	0.86	0.74

[a]NFNB - Net form net blotch, SFNB - Spot form net blotch
[b]*Pyrenophora teres* isolate
[c]Least significant differences (P=0.05)
[d]NA-Not applicable as there was only one replication

Chi-square analysis indicated single gene segregation for NFNB isolate WRS858 and two-gene segregation for NFNB isolate WRS1607 and SFNB isolate WRS857 (Table 5). The population segregated for three genes at the adult-plant stage in the field experiments, indicating the presence of adult-plant resistance genes which were not effective at the seedling stage. High correlations between different field experiment years (r =0.78 to 0.82) showed the consistency of natural disease epidemic and low inoculum variation across years.

Table 5. Chi-square analysis of CDC Bold/TR251 population reaction to net blotch

	Isolate/Test	# of R lines	# of S lines	R:S ratio	χ^2 Value	P value	# of genes*
NFNB	WRS858	64	86	1:1	3.23	0.07	One
	WRS1607	103	47	3:1	3.21	0.07	Two
	Field 2006	128	21	7:1	0.35	0.55	Three
	Field 2007	124	26	7:1	3.20	0.07	Three
	Field 2008	123	26	7:1	4.11	0.04	Three
SFNB	WRS857	119	31	3:1	1.50	0.22	Two
	LO233	15	135	1:7	0.86	0.35	Three

*Other gene ratios were evaluated and only those with the best fit are given here.

2.3 MEH #486/Harrington

One hundred and forty-eight lines (F8 generation) of this population were obtained from Dr. B. Legge, AAFC Brandon Research Centre, Brandon, MB. MEH #486 is a 2-row Ethiopian accession, resistant to NFNB isolates. The population was screened with isolates WRS858 (NFNB) and WRS857 (SFNB) at the seedling stage in the Phytotron and at the adult-plant stage in the field in 2006, 2007 and 2008.

MEH#486 showed resistance at the seedling and adult-plant stage and Harrington was susceptible (Table 6). High heritability in different experiments (0.83-0.93) indicates that phenotypic variability was mainly due to genetic factors. The population segregated for a single resistance gene for NFNB isolate WRS858 at the seedling stage (Table 7) and showed two-gene segregation for SFNB isolate WRS857. It segregated for three resistance genes at the adult plant stage to NFNB under natural infection in the field in 2006 and 2007 and for four resistance genes in the field in 2008.

Table 6. Net blotch reaction of parents of the MEH#486/Harrington population and checks

	NFNB				SFNB
Line/Isolate	WRS858	Field 2006	Field 2007	Field 2008	WRS857
MEH#486	2.7	1.8	1.8	2.0	2.8
Harrington	8.4	7.7	8.5	8.1	7.9
TR253	1.7	1.6	2.0	2.1	2.2
CDC Dolly	8.8	4.3	4.5	3.9	7.4
Popul Mean	5.3	3.7	3.3	3.2	6.2
ation Range	2.0-9.3	1.3-7.7	1.3-7.7	1.3-7.3	2.3-9.0
$LSD_{0.05}$	1.3	1.5	1.4	1.2	1.3
Heritability	0.93	0.83	0.88	0.87	0.90

Table 7. Chi-square analysis of MEH#486/Harrington population reaction to net blotch

	Isolate	# of R lines	# of S lines	R:S ratio	χ^2 Value	*P* value	# of genes*
NFNB	WRS858	71	77	1:1	0.24	0.62	one
	Field 2006	118	24	7:1	2.52	0.11	three
	Field 2007	121	21	7:1	0.68	0.41	three
	Field 2008	131	11	15:1	0.54	0.46	four
SFNB	WRS857	46	102	1:3	2.92	0.09	two

*Other gene ratios were evaluated and only those with the best fit are given here.

There was a good correlation (r=0.48-0.61) between adult-plant stage reaction in the field and seedling stage reaction to WRS858 in the Phytotron. However, at the adult plant stage a majority of the lines had reactions skewed towards resistance. The

correlation between adult-plant stage reaction in 2006, 2007 and 2008 was very high (r=0.73-0.80).

2.4 Damon/Harrington

This population was obtained from Dr. Brian Steffenson, Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota. Damon is a *H. spontaneum* barley accession from Israel, resistant to many diseases. Sixty-seven lines were evaluated against NFNB isolate WRS858 and SFNB isolate WRS857 at the seedling stage in the Phytotron. Damon was resistant to WRS858 and Harrington was susceptible (Table 8). To isolate WRS858, resistant and susceptible lines segregated into 27:40, not significantly different from a 1:1 ratio ($\chi^2 = 2.52$, P= 0.11) indicating a single resistance gene in Damon (Table 8). However, many lines showed intermediate reaction suggesting minor genes/modifiers for resistance to WRS858.

Table 8. Infection response of parental lines and checks to net blotch in Damon/Harrington population

Test/Line	Damon	Harrington	TR253	CDC Dolly	Population		Heritability	LSD
NFNB					Mean	Range		
WRS858	3.0	9.0	1.3	8.7	5.5	2.3-9.0	0.93	1.5
SFNB								
WRS857	NT	8.3	2.5	6.7	7.1	2.0-9.0	0.93	1.3

Table 9. Chi-square analysis of Damon/Harrington population reaction to net blotch

	Isolate	# of R lines	# of S lines	R:S ratio	χ^2 Value	P value	# of genes*
NFNB	WRS858	27	40	1:1	2.52	0.11	one
SFNB	WRS857	10	57	1:7	0.37	0.54	three

*Other gene ratios were evaluated and only those with the best fit are given here.

The population segregated for three genes for isolate WRS857 (Table 9). The majority of the lines were in the susceptible category for isolate WRS857. However, a few resistant lines were available which may be used as resistant sources. High heritability in all tests indicate that majority of phenotypic variation was due to genetic variation.

2.5 Shechem/Harrington

This population was also obtained from Dr. Brian Steffenson. Shechem, a *H. spontaneum* barley accession from Israel, showed resistance to many diseases. One hundred and three lines of this population were evaluated against NFNB isolate WRS858 and SFNB isolate WRS857 at the seedling stage. Shechem was highly resistant to WRS858 and Harrington was susceptible (Table 10). Similar to Damon/Harrington, this

population also segregated for a single gene for isolate WRS858 and for three genes for isolate WRS857 (Table 11).

Table 10. Infection response of parental lines and checks to net blotch in Shechem/Harrington population

Test/Line	Shechem	Harrington	TR253	CDC Dolly	Population		Heritability	LSD
NFNB					Mean	Range		
WRS858	1.3	8.3	1.8	8.7	4.9	2.0-9.0	0.93	1.5
SFNB								
WRS857	NT	8.2	2.5	6.3	7.5	3.0-9.0	0.91	1.3

Table 11. Chi-square analysis of Shechem/Harrington population reaction to net blotch

Isolate		# of R lines	# of S lines	R:S ratio	χ^2 Value	P value	# of genes*
NFNB	WRS858	64	39	1:1	6.07	0.01	One
SFNB	WRS857	11	91	1:7	0.27	0.60	Three

*Other gene ratios were evaluated and only those with the best fit are given here.

Dr. Steffenson's group is mapping the Damon/Harrington and Shechem/Harrington populations. QTL analysis will give us more power for better interpretation regarding number of genes/QTL involved in net blotch resistance and their chromosomal locations in these unique populations.

3. Evaluate and identify molecular markers linked to net blotch resistance

Several molecular markers linked to net blotch resistance have been reported from Australia (Raman et al. 2003, Cakir et al. 2003, Williams et al. 2003, Gupta et al. 2004). One sub-objective was to evaluate whether we can use Australian identified molecular markers in the western Canadian situation.

3.1 Can we use Australian identified molecular markers for net blotch resistance in western Canadian barley breeding programs?

The majority of parental lines of Australian barley mapping populations tested (11 of 18) were susceptible to western Canadian isolates and the rest showed intermediate reaction. Barley lines Franklin, Kaputar, Keel, Baudin and Hamelin (resistant to NFNB in Australia) were susceptible to the majority of the NFNB isolates tested indicating markers linked to their resistance may not be useful for Canadian breeding programs. Several workers reported that *P. teres* isolates from different regions/countries may differ in pathogenicity (Buchannon and McDonald 1965, Douiyssi et al. 1998, Gupta and Loughman 2001). Gupta and Loughman (2001) found 2 main virulences of *P. teres* f. *teres* in Western Australia which remained unchanged over a 20-year period whereas this pathogen was highly variable in Western Canada (Tekauz 1990). Australian lines WA4794, Pompadour, Halcyon, Tilga and Chebec showed a resistant reaction to some

Canadian isolates. Interestingly, CI9214, CI5791, TR251, Heartland, WPG8412 and WA4794 showed resistance to many Australian (Gupta et al. 2003, Gupta et al. 2006) as well as Canadian NFNB isolates (in this study) indicating that genes present in these lines could be effective against a wide range of isolates. Similarly, CI9214, TR251, Tilga and Keel showed resistance to many Australian (Williams et al. 2003, Gupta et al. 2006) as well as Canadian SFNB isolates.

3.2 Screening of Australian barley populations with Canadian net blotch isolates

Four Australian barley populations were screened at the seedling stage against specific Canadian net blotch isolates to which the Australian parents had shown differential reactions.

Materials and methods

Pompadour/Stirling: One hundred doubled-haploid (DH) lines were screened with NFNB isolates WRS102 and WRS1607 at the seedling stage in the Phytotron.

CI9214/Stirling: One hundred and two DH lines were evaluated at the seedling stage with NFNB isolate WRS858 and SFNB isolate WRS857 in the Phytotron.

Halcyon/Sloop: One hundred lines (84 spring type and 16 winter type) were screened at the seedling stage with NFNB isolate WRS858.

Chebec/Harrington: Eighty-one DH lines were obtained from Dr. J. K. Eglinton, Department of Plant Science, University of Adelaide, Glen Osmond, Australia. Chebec is a resistant South Australia barley cultivar. The population was screened at the seedling stage with SFNB isolate LO233. Molecular marker data for this population was obtained from Dr. J. K. Eglinton. Using 400 markers, a high-density genetic linkage map of the population was constructed and QTL analysis performed (for details see section 3.3).

In all experiments, TR253 (TR238//TR236/TR234), a highly resistant barley breeding line from AAFC Brandon, Manitoba, was used as a resistant check and Harrington was used as a susceptible check. CDC Dolly was used as a susceptible check if Harrington was a parent. Because of space constraints in the humidity chamber, each population was screened in two sub-experiments for each isolate. In each sub-experiment 50 DH lines, parents and checks (Harrington/CDC Dolly and TR253) were screened. Inoculum production, plant inoculation and disease assessment were as previously described.

Results and Discussion

Evaluation of the inheritance of resistance in four Australian populations against western Canadian *P. teres* isolates revealed one to three resistance genes depending upon isolate used. Similarly, other studies reported that net blotch resistance in barley was controlled by a single or several genes depending on the source of resistance, plant

development stage and pathotype (Ho et al. 1996; Steffenson et al. 1996; Alfanasenko et al. 1999; Manninen et al. 2000; Cakir et al. 2003; Williams et al. 2003). High heritability (0.86-0.95) revealed that phenotypic variance is mainly due to genetic effects. The Pompadour/Stirling population showed one and three gene segregation for NFNB isolates WRS1607 and WRS102, respectively. Cakir et al. (2003) and Gupta et al. (2004) reported single gene segregation in this population for several NFNB isolates. A strong correlation between disease reaction of WRS1607 and the Australian isolate 95B100 indicates that the same 6H gene/QTL was effective against isolate WRS1607. A major QTL, designated as *QRpt6*, was associated with seedling resistance for WRS1607 and WRS858 in the CDC Dolly/TR251 DH population (see section 3.3). *QRpt6* was also associated with adult-plant resistance in the field and validated in an unrelated MEH#486/Harrington population (section 3.4). This 6H QTL/gene is the most common locus contributing resistance to NFNB in barley (Steffenson et al. 1996; Manninen et al. 2000; Raman et al. 2003; Ma et al. 2004; Friesen et al. 2006; Gupta et al. 2006). SSR marker HVM74 (closely linked to *QRpt6*) and identified as part of this project, is now being used in molecular marker-assisted selection (MMAS) for net blotch resistance in the CDC barley breeding program.

The CI9214/Stirling population segregated for three resistance genes for NFNB isolate WRS858. Gupta et al. (2004) also reported three gene segregation for resistance to Australian NFNB isolates NB50 and NB52B, and mapped individual resistance genes on 6H, 4H, 3H and 2H depending on pathotype and plant development stage. QTL analysis identified two regions on chromosome 3H with a maximum R^2 value of 9% associated with seedling resistance to WRS858 (C. Li, personal communication). Similarly, Gupta et al. (2004) identified a major and minor gene on 3H associated with seedling resistance to NB50 and NB52B, respectively showing the same gene(s) is effective against some western Australian and a western Canadian isolate indicating the markers linked to this gene identified in Australia may be useful for MMAS in Canada for that Canadian isolate.

The CI9214/Stirling population showed single gene resistance for seedling SFNB resistance to isolate WRS857. Similarly, Williams et al. (2003) reported single gene (*Rpt4*) resistance to SFNB at the seedling stage in this population. However, the seedling stage reaction of this population to WRS857 and Australian isolates was not correlated ($r = -0.05$) indicating that *Rpt4* is not effective against WRS857. This was further confirmed by QTL analysis (C. Li, personal communication) of this population identifying a major QTL on 6H and minor QTL on 4H and 2H for reaction to isolate WRS857.

Halcyon was resistant (2.7) and Sloop susceptible (7.7) to NFNB isolate WRS858. Resistant and susceptible lines segregated 37:63 significantly different from 1:1 or 1:3 ratio indicating the seedling resistance in the Halcyon/Sloop population to isolate WRS858 was controlled by more than one gene. Raman et al. (2003) reported a major seedling resistance QTL on 4H (*QRpts4L*) in this population to Australian NFNB isolate NB50. The strong correlation ($r= 0.70$) between infection response for these two isolates indicates the same QTL is effective against WRS858. Interestingly, *QRpts4* was associated with seedling resistance to NFNB (WRS858) and SFNB (WRS857) in the

CDC Dolly/TR251 DH population (section 3.3). Raman et al. (2003) also reported a minor QTL on 6H (*QRpts6L*) in Halcyon/Sloop. Similarly, the frequency distribution of this population for WRS858 indicated the presence of minor genes/modifiers.

Chebec was resistant and Harrington susceptible to SFNB isolate LO233. The progeny of this population showed high heritability (0.93) for this reaction. Resistant and susceptible lines segregated 34:47 not significantly different from a 1:1 ratio ($\chi^2 = 2.09$, P=0.15) suggesting single gene resistance for isolate LO233 in this population. This was confirmed by QTL analysis, where a single major seedling resistance QTL mapped to chromosome 7H near PSR167b (Fig. 5), at the same position as *Rpt4* (Williams et al. 2003). However, *Rpt4* is of little practical value for the western Canadian barley breeding programs as it is not effective against SFNB isolate WRS857, the long-term standard Canadian SFNB isolate used to screen elite barley lines for SFNB resistance reaction.

In conclusion, this study reveals that molecular markers developed in one geographic region, may or may not be applicable in others due to the differences in germplasm and fungal pathogen strains used in different regions or countries. The majority of Australian mapping barley population parents (resistant to NFNB in Australia) were susceptible to Canadian net blotch isolates suggesting markers linked to their resistance will not be useful for the Canadian barley breeding programs. However, several net blotch resistance genes or QTL (*QRpts6*, *QRpts4*, 3H genes for NFNB and *Rpt4* for SFNB) were effective against both Australian and Canadian isolates indicating that molecular markers linked to these genes or QTL could be used for MAS in Canadian barley breeding programs. It is relatively faster and less expensive to validate identified molecular markers than to develop new ones. If applicable, these could be used for MMAS in local breeding programs. We found that marker HVM74 linked to *QRpt6* is useful for the CDC barley breeding program. However, it is also important to identify novel resistance genes effective against local pathogen populations and to develop molecular markers linked to these to allow efficient introgression/pyramiding into elite lines.

3.3 QTL mapping of net blotch resistance in CDC Dolly/TR251 using DArT

QTL analysis is useful for locating major and minor genes and determine their interactions in a segregating population. Most importantly, there is no need for arbitrary classification of lines into discrete resistance or susceptibility classes. QTL for net blotch resistance were mapped in the CDC Dolly/TR251 population using Diversity Arrays Technology (DArT®) markers.

Materials and Methods

DArT mapping and QTL analysis

DArT® markers were scored on the 90 DH lines by Triticarte Pty Ltd by probing genomic DNA from individual DH lines against the barley DArT® array. Based on hybridization signal intensities, 625 polymorphic markers were identified between the

parents and were scored on the DH mapping population as present or absent. Using 557 DArT® markers, a high-density genetic linkage map of the CDC Dolly/TR251 population was constructed using the Haldane mapping function within the software JoinMap 3.0 by employing logarithm of the odds (LOD) threshold values ranging from 3.0 to 5.0 (van Ooijen and Voorrips 2001). Linkage groups were initially separated into 10 groups on the basis of LOD score of 5.0. By comparing markers to the existing barley DArT and SSR/RFLP consensus map (Wenzl et al. 2006), the 10 linkage groups were regrouped into seven linkage groups corresponding to the seven barley chromosomes using LOD score of 3.0 or higher, and assigned chromosome names.

For QTL analysis, simple interval mapping (SIM) was performed on least square means to identify markers most significantly associated with variation in net blotch resistance. To enhance the power of QTL detection, the analyses was repeated using those markers identified by SIM as being significantly associated with net blotch resistance as co-factors for QTL in a multiple-QTL model (MQM) in MapQTL V 5.0. With MQM mapping, additional QTL were identified in some tests. QTL analysis was repeated by selecting markers associated with these QTL as co-factors as described by van Ooijen (2004). Permutation tests (1000 iterations) were performed for each experiment to determine the threshold at which the LOD score became significant ($P<0.05$) and highly significant ($P<0.001$) for QTL identification (van Ooijen 2004).

QTL nomenclature

'*QRptt*' indicates a QTL for resistance to *P. teres* f. *teres* (NFNB) and a '*QRptm*' indicates a QTL for resistance to *P. teres* f. *maculata* (SFNB). '*QRpt*' indicates a QTL for resistance to *P. teres* i.e. effective against both NFNB and SFNB. '*QRpt*' is followed by 's' or 'a' if effective only at seedling or adult plant stage and followed by the barley chromosome onto which the QTL was mapped.

Identification of SSR markers linked to net blotch resistance

Preliminary QTL analysis indicated a major NFNB seedling and adult plant resistance QTL on chromosome 6H and a major SFNB seedling resistance QTL on chromosome 4H. The parental lines were screened with 10 SSR markers previously mapped to chromosome 6H (surrounding the 6H QTL) and 17 SSR markers mapped to chromosome 4H to identify informative markers polymorphic between the parents. Four and seven polymorphic SSR markers from 6H and 4H, respectively were screened against the 90 DH lines.

For SSR screening, amplification was performed in a total volume of 25 µL, containing 100 µM each of dNTPs (Gibco BRL), 200 nM of each primer, 50 ng of genomic DNA, 2.0 mM of $MgCl_2$ (Gibco BRL), 1 unit of Taq polymerase and 1X of Gibco BRL buffer (50 mM KCL, 20 mM Tris-HCL, pH 8.4). Amplification was performed with a GeneAmp® PCR system 9700 (Applied Biosystems, USA) and consisted of an initial denaturation step at 94°C for 3 min, followed by 37 cycles as follows: a denaturation step at 94°C for 1 min, an annealing step at 55°C for 1 min and an

extension step at 72°C for 1 min; ending with an extension period of 72°C for 10 min. Amplified products were initially separated on 2% agarose, 0.5 X TBE gel, stained with ethidium bromide and viewed under ultra-violet light. Amplified products were then separated on 6% polyacrylamide gels and viewed with silver staining if polymorphism was not visible on agarose gels. The 11 SSR markers were placed on the DArT® map using JoinMap 3.0. QTL analysis was performed with MapQTL V 5.0 as described earlier.

QTL effect on net blotch resistance

To determine the effect of individual QTL and their combinations on net blotch resistance, the mean IR of all lines with the resistance allele for each QTL or their combinations was calculated and compared with that of lines having the susceptible allele for that particular QTL. A T-test ($P = 0.05$) was performed to determine statistical significance. For NFNB resistance, IR of lines with *QRpt6* were compared with IR of lines having combinations of *QRpt6* and other QTL or no QTL using a T-test.

Results and Discussion

Triticarte® scored 625 DArT® markers on 94 barley DNA samples with a call rate of 95 %. A high density genetic linkage map was constructed using 557 mapped DArT® and 11 SSR markers. Polymorphism information content (PIC) values of the 568 identified polymorphic markers ranged from 0.33-0.50, with an average of 0.49. Chromosome 7H had the largest number of markers (141), while chromosome 4H had the lowest (27). The genetic linkage map spanned a distance of 925 cM (Table 12) with an average two-locus interval of 1.6 cM. Chromosome sizes ranged from 94 cM (2H) to 172 cM (5H). There were few large gaps (> 20 cM) between markers; one each for chromosomes 1H and 3H and two for 4H. Large gaps on 4H have been found in other studies (Qi et al. 1996; Karakousis et al. 2003b; Kleinhofs et al. 1993; Wenzl et al. 2006), indicating 4H is less polymorphic for DArT and other molecular markers.

A NFNB seedling resistance QTL- *QRpt6* spanning an approximately 1 cM interval on chromosome 6H was identified for isolates WRS858 and WRS1607 (Fig. 2). The same QTL was identified for adult-plant resistance in 2005 and 2006 field trials. These results confirm earlier reports of a major QTL or gene on chromosome 6H (Steffenson et al. 1996; Manninen et al. 2000; Cakir et al. 2003; Gupta et al. 2004; Friesen et al. 2006). Three additional QTL for seedling resistance to the more virulent NFNB isolate WRS858 were identified on chromosomes 2H, 4H and 5H (Table 13). A significant adult-plant resistance QTL - *QRpta5* was also identified on chromosome 5H from the 2005 field trial. This may be due to higher natural infection in 2005 versus the 2006 field trial. Major/minor QTL for NFNB resistance on 2H (Steffenson et al. 1996; Richter et al. 1998; Cakir et al. 2003; Raman et al. 2003; Ma et al. 2004), 4H (Steffenson et al. 1996; Richter et al. 1998; Spaner et al. 1998; Raman et al. 2003; Yun et al. 2005) and 5H (Steffenson et al. 1996; Spaner et al. 1998) have been reported. These results

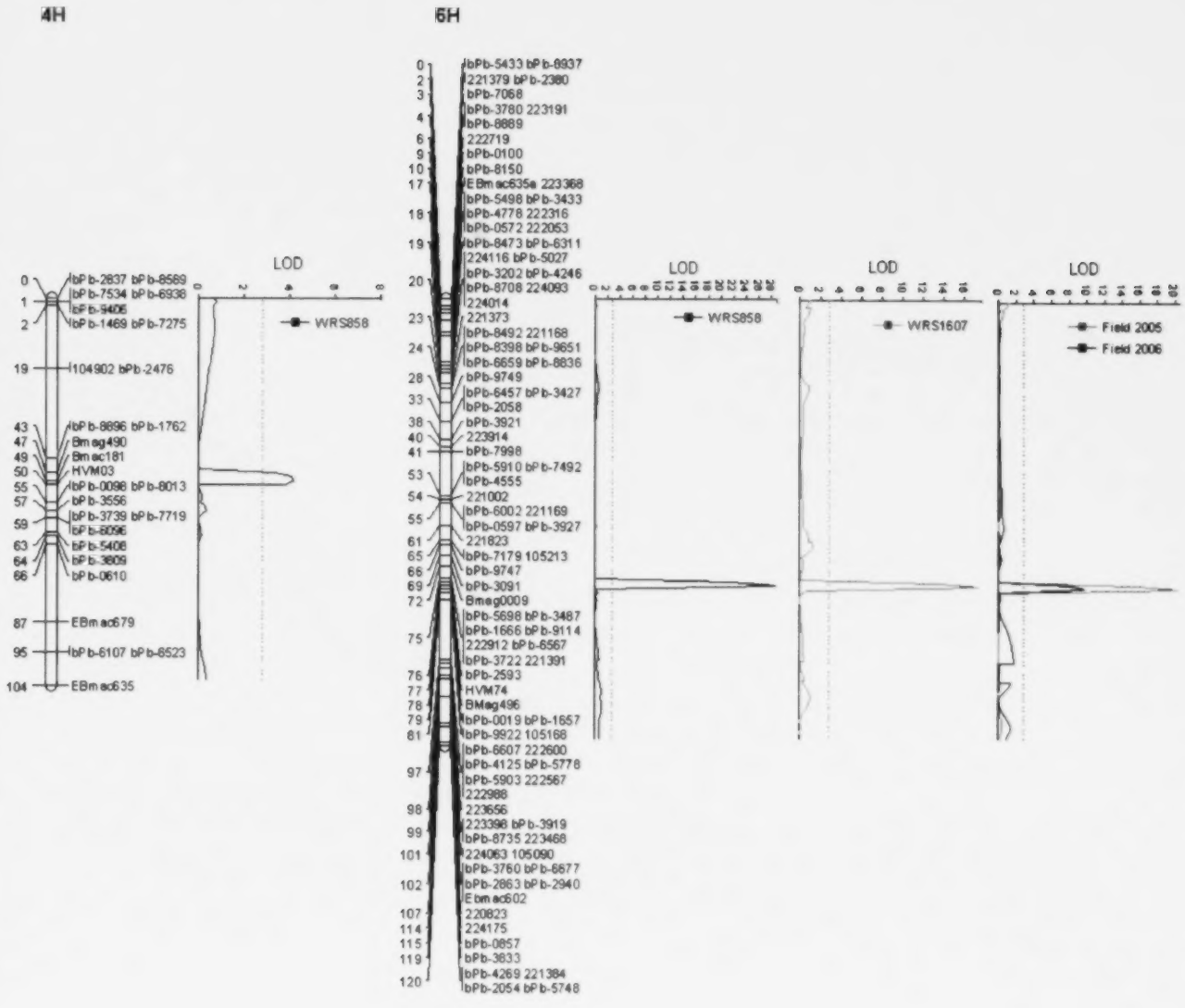

Fig. 2. Multiple-QTL model (MQM) LOD scans of chromosomes where QTL were detected for NFNB resistance in the CDC Dolly/TR251 DH population. Vertical lines indicate significance threshold for each experiment, estimated from 1000 permutations of the data. One QTL on 4H associated with NFNB (WRS858) resistance. A major QTL on 6H associated with seedling (WRS858, WRS1607) and adult-plant (Field 2005, field 2006) resistance. (Two additional QTL on 2H and 5H associated with seedling resistance to WRS858 and a adult-plant resistance QTL on 5H for field 2005 not shown.)

suggest the CDC Dolly/TR251 population is segregating for the majority of resistance QTL thus the population could be useful for fine mapping or candidate gene validation.

A highly significant seedling resistance QTL-*QRpts4* for the SFNB isolate WRS857 was detected on chromosome 4H and a significant QTL- *QRpt7* mapped to chromosome 7H (Table 13). Williams et al. (1999) mapped a major seedling resistance gene *Rpt4*/QTL, explaining 80% of SFNB reaction variation, on chromosome 7H in cv 'Galleon' and the same gene was responsible for SFNB seedling resistance in five other barley lines (Williams et al. 2003). Based on the consensus map (Wenzl et al. 2006), *QRpt7* in the CDC Dolly/TR251 population is at the same location as *Rpt4*. The major SFNB seedling resistance QTL - *QRpts4* identified in this study is novel. Interestingly, Williams et al. (2003) detected adult-plant resistance QTL on chromosome 4H in VB9104 linked to SSR marker HVM03, but this QTL was not effective at the seedling stage. This suggests that *P. teres* f. *maculata* populations in Canada and Australia are different. Isolate WRS857, used in this study, is a long-term standard Canadian SFNB isolate used to screen elite barley lines for SFNB resistance reaction. Recently, Friesen et al. (2006) reported a major SFNB seedling resistance QTL on 4H, explaining 64% of the phenotypic variance. However, the position of that QTL (near the 4H telomere) is not the same as that of the QTL reported here.

Table 13. QTL identified for net blotch resistance in the CDC Dolly/TR251 population

Test	QTL	Chr	Support interval	Closest marker[b]	LOD	r^2 [c]	Add. effect[d]
[a]NFNB							
WRS858	*QRpt6*	6H	76-77 cM	HVM74	28.9**	65	1.30 (TR251)
	QRptts2	2H	63-65 cM	bPb-9925	6.8**	8	0.46 (TR251)
	QRpts4	4H	47-50 cM	Bmac181	4.2**	5	0.34 (TR251)
	QRptts5	5H	109 cM	bPb-8462	5.0**	6	0.38 (CDC Dolly)
WRS1607	*QRpt6*	6H	76-77 cM	HVM74	17.7**	60	0.86 (TR251)
Field 2005	*QRpt6*	6H	76-77 cM	HVM74	20.6**	60	0.92 (TR251)
	QRptta3	3H	116-117 cM	bPb-9599	2.8	5	0.27 (TR251)
	QRptta5	5H	92-103 cM	bPb-1462	3.4*	6	0.29 (TR251)
	QRpt7	7H	130-133 cM	222163	2.8	5	0.27 (TR251)
Field 2006	*QRpt6*	6H	76-77 cM	HVM74	9.7**	39	0.52 (TR251)
SFNB							
WRS857	*QRpts4*	4H	49-55 cM	HVM03	5.3**	20	0.52 (TR251)
	QRpt7	7H	125-133 cM	222163	3.3*	12	0.42 (TR251)
	QRpt6	6H	76-77 cM	HVM74	1.9	6	0.30 (TR251)

*Significant ($P < 0.05$); **highly significant ($P < 0.001$).

[b]Marker with maximum LOD (logarithm of the odds).

[c]The amount of total trait variance explained by a QTL at this locus, as %.

[d]Additive effect for QTL association and name given in bracket is the parent contributing resistance allele at the QTL.

Table 14. Average infection response of lines with NFNB seedling and/or adult-plant resistance QTL from the CDC Dolly/TR251 population

	[a]WRS858	[a]WRS1607	Field 2005	Field 2006	[c]# of lines
Seedling resistance QTL					
[b]*QRpt6*	4.3	3.0	3.4	2.3	51
QRpt6+QRptts2	3.6**	3.0	3.3	2.3	27
QRpt6+QRptts2+QRpts4	3.4**	2.9	3.3	2.2	12
QRpt6+QRptts2+QRpts4+QRptts5	2.8**	2.8	3.7	2.3	7
No SR QTL	7.5**	4.9**	4.3	3.5**	3
Adult-plant resistance QTL					
QRpt6+QRptta5	4.7	3.2	2.9**	2.2	20
QRpt6+QRptta5+QRptta3	4.4	3.0	2.7**	2.2	8
QRpt6+QRptta5+QRptta3+QRpt7	4.5	3.4	2.9	1.9	4
No APR QTL	6.5**	5.1**	6.2**	3.6**	4

[a]*Pyrenophora teres* isolate.
[b]Least square means of lines with *QRpt6* in each test were compared with that of lines with QTL combinations using T-test.
[c]Total number of lines with resistance allele at particular QTL or their combinations.
**Highly significant ($P < 0.01$) than the value in the first column when read in a respective column.

DArT markers generally cannot easily be used for routine MMAS, thus there is a need to identify markers linked to highly significant QTL, which could be used for routine MAS. SSR markers are an obvious choice, as they show high polymorphism, are co-dominant, usually chromosome-specific, reliable and simple to assay. Using a DArT/SSR consensus map, we were able to identify SSR markers linked to the major NFNB and SFNB resistance QTL. HVM74 was the closest marker (1 cM) to the NFNB seedling and adult-plant resistance QTL *QRpt6* and flanked by SSR markers Bmag496 (1 cM) and Bmag9 (4 cM). Similarly, HVM03 was the closest marker (5 cM) to the SFNB seedling resistance QTL - *QRpts4* and two other markers (Bmac181 and Bmag490) were also linked.

Three QTL (*QRpt6*, *QRpts4*, and *QRpt7*) were effective for resistance to both forms of net blotch. Lines selected based on *QRpt6* or its combination with two other markers had better resistance to both NFNB and SFNB (Table 14). For the most part, epistatic interaction between QTL were not significant, suggesting largely additive effects. The major seedling and adult-plant resistance QTL, *QRpt6* may be sufficient to provide adequate field resistance to NFNB (Table 15) and could be selected for on the basis of SSR markers. High heritability for different tests indicated that selection based on molecular markers is feasible. This was validated in an unrelated barley population MEH#486/Harrington where *QRpt6* alone provided better resistance to both forms of net blotch. *QRpt6* in combination with *QRpts4* showed better resistance than *QRpt6* alone, but differences were not significant confirming the epistatic interaction between different QTL was not significant. We propose MMAS for *QRpt6* using SSR markers for NFNB resistance, which could be combined with *QRpts4* if SFNB is also important.

Table 15. Average infection response of lines with or without net blotch resistance QTL from the CDC Dolly/TR251 population

QTL/Test	[b]Allele	[a]*NFNB*				**SFNB**	
		[c]WRS858	[c]WRS1607	Field 2005	Field 2006	[c]WRS857	[d]# of lines
QRpt6	R	4.2**	3.0**	3.4**	2.3**	6.3	51
	S	6.8	4.8	5.1	3.3	6.7	39
QRpts4	R	5.2	3.8	4.3	2.7	6.0**	48
	S	5.5	3.8	3.9	2.7	7.0	42
QRpt7	R	5.9	3.9	3.9	2.6	6.0**	28
	S	5.2	3.8	4.3	2.8	6.7	56
QRpt6+QRpts4	R	4.0**	2.9**	3.5**	2.3**	5.6**	25
	S	7.1	4.7	4.8	3.5	7.1	16
QRpt6+QRpt7	R	4.4**	2.8**	3.2**	2.0**	5.3**	14
	S	6.6	4.7	5.5	3.5	6.8	22
QRpts4+ QRpt7	R	5.4	3.7	3.8	2.2*	5.1**	15
	S	5.1	3.6	4.0	2.6	7.1	27
QRpt6+ QRpts4+QRpt7	R	4.4**	2.8**	3.0**	1.9**	4.5**	9
	S	6.8	4.7	5.4	3.5	7.5	7

[a]NFNB - Net form net blotch. SFNB - Spot form net blotch

[b]Resistance (R) or susceptible (S) allele at a particular QTL locus/loci.

[c]*Pyrenophora teres* isolate

[d] Total number of lines with R or S allele at particular QTL.

*Significant (P <0.05); **highly significant (P <0.01) using T-test between genotypic classes at particular QTL or their combinations.

3.4 Validation of SSR markers in MEH #486/Harrington population

The MEH#486/Harrington population was selected for validation of SSR markers HVM74 and HVM03 linked to the 6H QTL for NFNB resistance and to the 4H QTL for SFNB resistance, respectively. These lines were screened with SSR markers HVM74 and HVM03 and scored on agarose gel. The population was screened with NFNB isolate WRS858 and SFNB isolate WRS857 at the seedling stage in the Phytotron (see section 2.5). The population was also screened at the adult-plant stage in the field at Melfort in 2006, 2007 and 2008.

MEH#486 exhibited resistance at the seedling and adult-plant stage and Harrington was susceptible (Table 6). Allelic variation at HVM74, linked to *QRpt6*, was associated with MEH #486 seedling resistance to NFNB isolate WRS858 and adult-plant resistance in the field (Table 16), indicating *QRpt6* is present in MEH#486. Similarly, HVM03 linked to *QRpts4* was associated with SFNB resistance in this population. Lines with the MEH#486 allele at the *QRpt6* locus had an IR of 4.0 at the seedling stage compared with 6.8 for lines with the Harrington allele. At the adult-plant stage in field in 2006, lines with the MEH#486 allele had an IR of 3.0, whereas lines with the Harrington allele had an IR of 4.5. Similar results were observed in field in 2007 and 2008. Lines with *QRpt6* showed significantly better resistance to both forms of net blotch than those without *QRpt6*.

Lines with *QRpts4* showed lower IR than those without *QRpts4* but the difference was non-significant except in the field 2007 experiment where IR was significantly lower with *QRpts4*. Selection based on both markers showed better resistance than either or no marker, but the differences were non-significant as compared to lines with *QRpt6* alone except for isolate WRS858 (Table 16), further proving selection based on *QRpt6* alone could give better resistance to both net blotch forms.

Table 16. Average infection response of lines from the MEH#486/Harrington population with or without major net blotch resistance QTL (*QRpt6*, *QRpts4*)

QTL/Test	[b]Allele	NFNB				SFNB	[d]# of lines
		[c]WRS858	Field 2006	Field 2007	Field 2008	[c]WRS857	
QRpt6	R	4.0**	3.0**	2.7**	2.8**	5.7**	79
	S	6.8	4.5	4.0	3.8	6.8	69
QRpts4	R	5.0	3.6	3.0*	3.2	6.0	62
	S	5.6	3.8	3.6	3.4	6.4	83
QRpt6+QRpts4	R	3.4**	2.8**	2.4**	2.8**	5.4**	25
	S	7.1	4.8	4.5	4.0	6.9	32

[b] Resistance (R) or susceptible (S) allele at a particular QTL locus/loci.
[c] *Pyrenophora teres* isolate
[d] Total number of lines with R or S allele at particular QTL.
*Significant (P <0.05) or **highly significant (P <0.01) using T-test at particular QTL or their combinations.

3.5 QTL mapping of net blotch resistance in CDC Bold/TR251 population

One hundred and fifty DH lines were screened at the seedling stage with NFNB isolates WRS858 and WRS1607 and SFNB isolates WRS857 and LO233 in the Phytotron and at the adult-plant stage in the field in 2006, 2007 and 2008 at the CDC NFNB nursery at Melfort, SK. Dr. Ravi Chibbar's group at the Department of Plant Sciences, University of Saskatchewan mapped 89 DH lines of this population using 680 DArT, AFLP and SSR markers. We have net blotch reaction of 75 lines at the seedling and adult-plant stage. The CDC Bold/TR251 map was used to identify QTL associated with NFNB resistance in TR251 and compare with those identified from the CDC Dolly/TR251 population. QTL mapping was performed as described earlier (see section 3.3).

A NFNB seedling resistance QTL- *QRpt6* spanning an approximately 1 cM interval on chromosome 6H was identified for isolates WRS858 and WRS1607 (Fig. 3). The same QTL was identified for adult-plant resistance in 2006, 2007 and 2008 field trials (Table 17).

Table 17. QTL identified for net blotch resistance from CDC Bold/TR251 population

Test	QTL	Chrom.	Support interval	Closest marker[a]	LOD	r^{2}[b]	Add. effect[c]
NFNB							
WRS858	*QRpt6*	6H	63.4-63.5	HVM62b	16.3**	48	1.36(TR251)
	QRptts1.1	1H	17.8-22.9	E32M48.4	5.8*	12	-0.67(Bold)
WRS1607	*QRpt6*	6H	63.4-63.5	HVM62b	14.3**	53	1.39(TR251)
	QRptts1.2	1H	52.4-56.8	E38M59.8	3.2*	8	-0.53(Bold)
Field 2006	*QRpt6*	6H	63.4-63.5	HVM62b	22.3**	61	1.70(TR251)
	QRptta2	2HL	19.6-27.3	BPb5942	3.7*	5	0.50(TR251)
	QRptta5.1	5H	112.1-120.5	E35M49.5	3.6*	5	0.48(TR251)
	QRptta5.2	5H	200.4-206.8	E33M47.7	3.5*	5	-0.47(Bold)
Field 2007	*QRpt6*	6H	63.4-63.5	HVM62b	13.5**	54	1.28(TR251)
	QRptta5.2	5H	199.4-206.8	E33M47.7	4.1*	12	-0.59 (Bold)
Field 2008	*QRpt6*	6H	66.2-66.7	E33M61.2	9.8**	32	0.93(TR251)
	QRptta5.1	5H	112.1-120.5	E35M49.5	4.5*	12	0.59(TR251)
SFNB							
WRS857	*QRptms1*	1H	139.1-151.8	Bmag579	4.2*	14	0.50(TR251)
	QRptms4	4H	25.8-38.4	Ebmac788	3.9*	13	0.48(TR251)
	QRptms6	6H	52.6-55.0	E35M61.9	6.2**	22	0.65(TR251)
LO233	*QRptms2*	2HL	0.0-1.1	bPb1051	3.5*	18	0.40(TR251)
	QRptms3	3H	94.1-96.8	E37M50.7	2.7	13	-0.35(Bold)

*Significant ($P < 0.05$); **highly significant ($P < 0.001$).

[a]Marker with maximum LOD.

[b]The amount of total trait variance explained by a QTL at this locus, as %.

[c]Additive effect for QTL association and name given in bracket is the parent contributing for the QTL.

In addition to the major QTL *QRpt6*, other QTL were also identified. Seedling resistance QTL *QRptts1.1* and *QRptts1.2* were identified for NFNB isolates WRS858 and WRS1607, respectively (Table 17). Both QTL were contributed by the susceptible parent CDC Bold. Three additional adult-plant resistance QTL (*QRptta2*, *QRptaa5.1*, *QRptta5.2*) were identified from Field 2006 data and one (*QRptta5.2*) from Field 2007 and one (*QRptaa5.1*) from field 2008. This suggests some QTL were effective only at the seedling stage while others were effective only at the adult-plant stage. The major NFNB seedling and adult-plant resistance QTL *QRpt6* was the same as identified in CDC Dolly/TR251 but the additional QTL were different from those identified from the CDC Dolly/TR251 population.

For SFNB isolate WRS857, three QTL were identified; one each on 1H (*QRptms1*), 4H (*QRptms4*) and 6H (*QRptms6*) explaining 49% phenotypic variation (Table 17). All these QTL are contributed by the resistant parent, TR251. Interestingly, these QTL were different than what those found for the CDC Dolly/TR251 population with this isolate. The 4H QTL was identified in CDC Dolly/TR251 but it is in a different position in the CDC Bold/TR251 population. A non-significant QTL on 6H was identified from CDC Dolly/TR251, however, the 6H QTL (*QRptms6*) is highly significant for CDC Bold/TR251 but again at a different chromosomal position. *QRpt7*, identified in CDC Dolly/TR251, is not present in the CDC Bold/TR251 population. A significant QTL on 2HL (*QRptms2*) explaining 18 % phenotypic variation was identified for SFNB isolate LO233 in CDC Bold/TR251 and a non-significant QTL on 3H (*QRptms3*) was also identified.

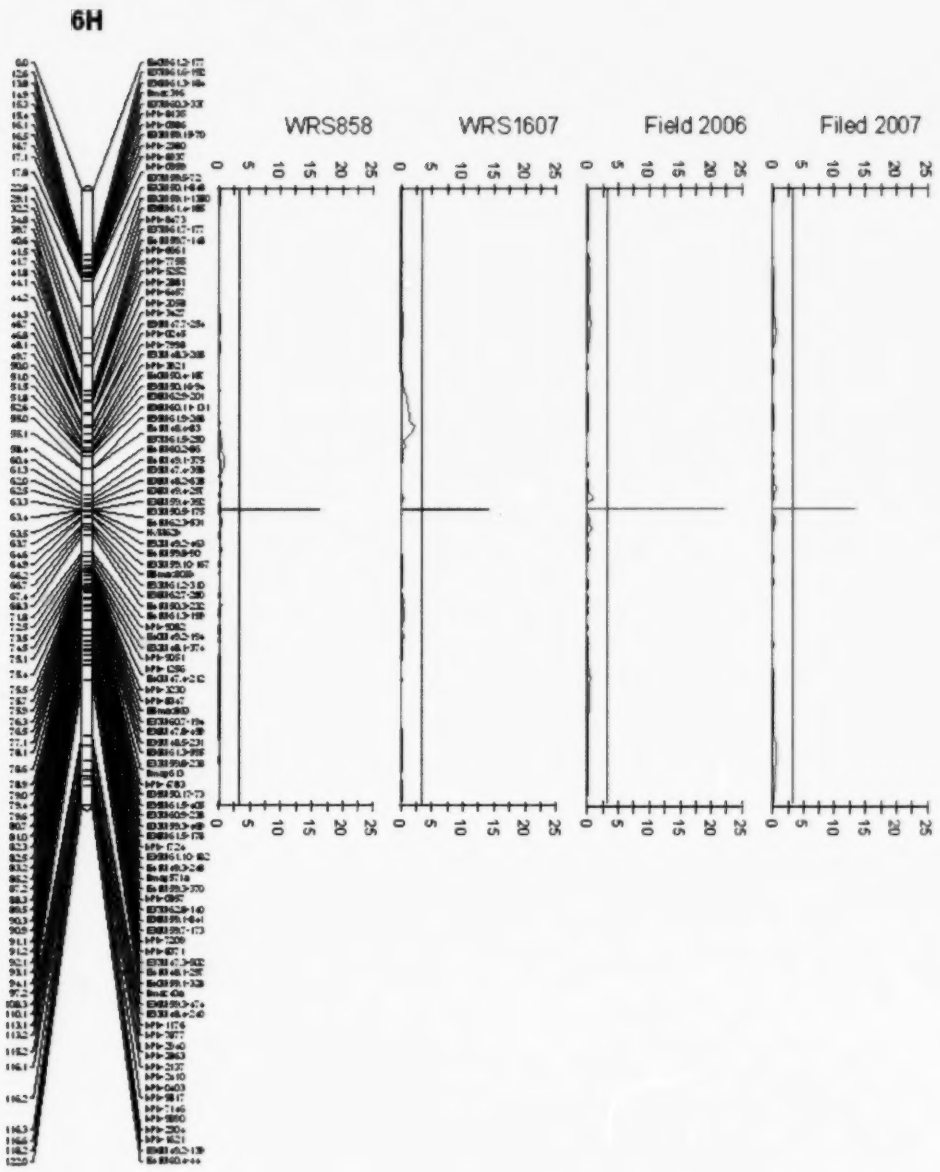

Fig. 3. Multiple-QTL model (MQM) LOD scans of chromosome 6H where a major QTL associated with seedling (WRS858, WRS1607) and adult-plant (Field 2006, field 2007) resistance was detected for NFNB resistance in the CDC Bold/TR251 DH population. Vertical lines indicate significance threshold for each experiment, estimated from 1000 permutations of the data.

4.0 Utilization of identified molecular markers for MMAS

The parents of barley populations available in the CDC barley breeding program, which are known to be segregating for net blotch resistance, were screened with the SSR markers linked to the 6H QTL for NFNB resistance and to the 4H QTL for SFNB resistance for their practical utilization.

Screening the parents of 22 barley populations with SSR markers linked to *QRpt6* and *QRpts4* identified many polymorphic populations (Table 18). SSR markers HVM74 and Bmag9, linked to *QRpt6*, were polymorphic between parents of seven populations whereas Bmag496 was polymorphic between parents of 12 populations. SSR marker HVM03, linked to *QRpts4*, was polymorphic between parents of 19 populations. Interestingly, the HVM03 polymorphism was detected on agarose gel for all except Valier × TR244 which was polymorphic on polyacrylamide gel. Bmac181 and Bmag490 showed polymorphism for ten and eight populations, respectively. The results indicate these SSR markers could be used for practical MMAS in many barley populations available in the CDC barley breeding program. Four barley populations were screened with SSR markers HVM74 and HVM03 to select lines resistant to both forms of net blotch. The SSR screening protocol was as described in section 3.3.

McLeod/CDC Helgason: One hundred and forty-six lines were screened with HVM74 and HVM03. To HVM74, 64 lines showed the resistance allele of CDC Helgason and 79 showed the susceptible allele from McLeod and 3 were heterozygous. Seventy-one lines exhibited the resistance allele for HVM03 and 67 lines showed the susceptible allele. Based on the genotyping with both markers, 31 resistant lines showed resistance alleles for both markers.

The population was screened with NFNB isolate WRS858 and WRS102 and SFNB isolate WRS857 at the seedling stage in the Phytotron (see section 2.5). CDC Helgason was resistant and McLeod was susceptible to all the isolates (Table 19). The reaction of resistant and susceptible check as expected. High heritability for different tests (0.92-0.98) revealed that the majority of the phenotypic variance was due to genetic effects. The population segregated for a single resistance gene for NFNB isolate WRS102 and SFNB isolate WRS857 (Table 20). However, for NFNB isolate WRS858, the population didn't segregate for a specific gene-model ratio.

Allelic variation at HVM74, linked to *QRpt6*, was associated with CDC Helgason seedling resistance to NFNB isolate WRS858 and WRS102 (Table 21), indicating *QRpt6* is present in CDC Helgason. Similarly, HVM03 linked to *QRpts4* was associated with SFNB resistance in this population.

Table 18. Screening parents of barley populations segregating for net blotch resistance with SSR markers linked to resistance QTL

Barley populations	Population type[b]	Polymorphic for HVM74[c]	Bmag496[c]	Bmag9[c]	HVM03[d]	Bmac181[d]	Bmag490[d]
MEH#486[a] × Harrington	F_8	Yes	Yes	Yes	Yes	No	Yes
Valier × **TR253**	DH	No	No	No	Yes	Yes	Yes
HB348 × **TR253**	DH+RILs	No	Yes	No	Yes	No	No
HB328 × **TR244**	DH	No	Yes	No	Yes	Yes	Yes
CDC Bold × **TR251**	DH	Yes	Yes	Yes	Yes	No	No
CDC McGwire × **TR251**	DH	No	No	No	No	No	No
CDC McGwire × TR640	RILs	No	No	No	Yes	No	No
CDC Helgason × McLeod	RILs	Yes	Yes	Yes	Yes	Yes	Yes
KXN/TLN-147 × **CDC Helgason**	F_5	Yes	Yes	Yes	Yes	No	No
WI3350 × **CDC Helgason**	F_5	No	Yes	No	Yes	Yes	No
LG15/PTY//BHM-067 × **TR02367**	F_5	No	Yes	No	Yes	Yes	No
Rangoon × **TR03375**	F_5	No	Yes	No	Yes	Yes	Yes
RCSL-096 × **TR03375**	F_5	No	No	No	Yes	No	Yes
WI3350 × **CDC McGwire**	F_5	No	Yes	No	Yes	Yes	No
SB01045 × **TR02367**	F_5	Yes	No	Yes	Yes	Yes	Yes
TR03374 × **TR02367**	F_5	No	No	No	No	No	No
SH00243 × **CDC McGwire**	F_5	No	Yes	No	Yes	Yes	No
KM2062 × **CDC McGwire**	F_5	No	No	No	Yes	No	No
XB2149-N96 × **CDC McGwire**	F_5	No	No	No	Yes	No	No
CDC Bold × McLeod	RILs	Yes	No	Yes	Yes	Yes	Yes
CDC McGwire × CDC Bold	RILs	Yes	Yes	Yes	Yes	No	No
Manley × **TR244**	DH	No	No	No	No	No	No

[a]Parents in bold are resistant to net blotch.
[b]Population types : DH - Doubled-haploid; RILs - recombinant inbred lines.
[c]SSR marker linked to the major NFNB resistance QTL on 6H, *QRpt6*.
[d]SSR marker linked to the major SFNB resistance QTL on 4H, *QRpts4*.

Table 19. Infection response of parental lines and checks to net blotch in McLeod/CDC Helgason population

Line/Test		[a]NFNB		SFNB
		[b]WRS858	[b]WRS102	[b]WRS857
McLeod		8.4	7.4	6.9
CDC Helgason		3.1	1.9	2.1
TR253		1.9	1.1	2.4
Harrington		8.4	7.9	8.4
Popul ation	Mean	5.8	4.7	5.7
	Range	2.0-9.0	1.0-9.0	2.0-9.0
[c]$LSD_{0.05}$ lines		1.3	1.1	1.3
$LSD_{0.05}$ lines vs. checks		1.0	0.9	1.0
Heritability		0.96	0.98	0.92

[a]NFNB - Net form net blotch, SFNB - Spot form net blotch
[b]*Pyrenophora teres* isolate
[c]Least significant differences (P=0.05)

Table 20. Chi-square analysis of McLeod/CDC Helgason population reaction to net blotch

	Isolate	# of R lines	# of S lines	R:S ratio	χ^2 Value	P value	# of gene(s)*
NFNB	WRS858	56	90	Does not fit any specific gene ratio			
	WRS102	74	72	1:1	0.03	0.86	one
SFNB	WRS857	60	86	1:1	4.63	0.03	one

*Other gene ratios were evaluated and only those with the best fit are given here.

Lines with the CDC Helgason allele at the *QRpt6* locus had an IR of 3.9 and 2.7 at the seedling stage compared with 7.3 and 6.3 for lines with the McLeod allele for isolates WRS858 and WRS102, respectively (Table 21). Interestingly, lines with the *QRpt6* allele had significantly lower reaction to SFNB isolate WRS857 than without *QRpt6* allele. In conclusion, lines with *QRpt6* showed significantly better resistance to both forms of net blotch than those without *QRpt6*.

Lines with *QRpts4* showed significantly lower IR for SFNB isolate WRS857 than those without *QRpts4* but the difference was non-significant for NFNB isolates WRS858 and WRS102. Selection based on marker HVM74 (*QRpt6*) showed better resistance than both or no marker, further proving selection based on *QRpt6* alone could give better resistance to both net blotch forms.

Table 21. Average infection response of lines from the McLeod/CDC Helgason population with or without major net blotch resistance QTL (*QRpt6*, *QRpts4*)

QTL/Test	[b]Allele	NFNB [c]WRS858	NFNB [c]WRS102	SFNB [c]WRS857	[d]# of lines
QRpt6	R	3.9**	2.7**	5.2**	64
	S	7.3	6.3	6.1	79
QRpts4	R	5.9	4.7	5.3**	71
	S	5.8	4.9	6.2	67
QRpt6+*QRpts4*	R	4.3**	2.9**	4.8**	31
	S	7.5	6.8	6.7	37

[b] Resistance (R) or susceptible (S) allele at a particular QTL locus/loci.
[c] *Pyrenophora teres* isolate
[d] Total number of lines with R or S allele at particular QTL.
**Highly significant (P <0.01) using T-test at particular QTL or their combinations.

KXN/TLN147 x CDC Helgason: Fifty-eight lines were screened with HVM74 and HVM03. CDC Helgason was resistant to NFNB isolate WRS858 and SFNB isolate WRS857 whereas KXN/TLN147 was susceptible at the seedling stage in the Phytotron (Table 22). Seven lines were selected with markers for both resistance alleles and 18 lines without marker alleles were selected.

Table 22. Screening of barley parental lines with NFNB isolate WRS858 and SFNB isolate WRS857 at the seedling stage in the Phytotron and at the adult-plant stage in the field 2007 and 2008

	WRS858	WRS857	Field 2007	Field 2008
CDC Helgason	2.7	2.0	2.1	2.8
KXN/TLN147	6.7	6.3	3.0	3.8
SB01045	8.0	5.0	2.5	3.1
TR02367	1.7	1.3	2.1	2.3
McLeod	8.7	6.3	3.0	NT
CDC Bold	6.7	5.0	6.3	5.4
Harrington	8.5	8.2	8.5	8.0
TR253	2.0	2.2	2.0	2.0
Kao	NT*	NT	1.0	1.0

*Not tested.

SB01045/TR02367: TR02367 was resistant and SB01045 was susceptible to isolates WRS858 and WRS857 at the seedling stage (Table 22). Of 59 lines screened, 37 and 26 lines showed the resistance allele for HVM74 and HVM03, respectively. Sixteen lines exhibiting both resistance alleles and eight lines without alleles were selected.

McLeod/CDC Bold: Two hundred and fifty-six lines were screened with both the markers. This population was selected as CDC Bold showed intermediate reaction to

many isolates at the seedling stage in the Phytotron. Thirty-two lines were selected with the CDC Bold allele.

Field screening of MAS selected lines

All MMAS selected resistant lines and some susceptible lines (based on the markers) from the three barley population were planted in the CDC NFNB disease screening nursery at Melfort, SK during the summer of 2007. There were three replications of each line. Parental lines and checks (Harrington, TR251, Kao 32-12) were widely distributed. NFNB disease incidence was lower in 2007 and most parental lines showed a resistant reaction at the adult-plant stage (Table 22). Secondly, these parental lines may have adult-plant resistance genes. There was no segregation of disease reaction on tested lines. Therefore, we could not determine the effect of MMAS based on the field 2007 experiment.

The selected lines from all three populations were screened at the seedling stage with NFNB isolate WRS858 and SFNB isolate WRS857 in the Phytotron to evaluate if MMAS selected resistant lines perform better than MMAS selected susceptible lines and to determine if the putative resistant lines selected by MMAS show similar reactions as of their respective resistant parent. The selected lines from KXN/TLN 147/CDC Helgason and SB01045/TR02367 populations were also screened at the adult-plant stage in the field at Melofrt in 2008.

From the KXN/TLN 147/CDC Helgason population, MMAS resistant lines showed significantly lower disease reaction than MMAS susceptible lines at the seedling stage for NFNB isolate WRS858 and at the adult-plant stage in the field 2008 (Table 23). However, to SFNB isolate WRS857, the disease reaction was not significantly different between resistant and susceptible lines.

Table 23. Average infection response of lines from the barley populations with or without major net blotch resistance QTL (*QRpt6* and *QRpts4*)

Population	[b]Allele	NFNB		SFNB	[d]# of lines
		[c]WRS858	Field 2008	[c]WRS857	
KXN/TLN	R	3.7**	2.7*	4.2	7
147/CDC Helgason	S	7.6	3.2	5.1	18
SB01045/TR02367	R	3.4**	2.5**	2.9**	16
	S	6.1	3.5	6.0	8
CDC Bold/McLeod	R	5.4	NT	5.9	32
	S	5.2	NT	6.0	17

[b] Resistance (R) or susceptible (S) allele at *QRpt6* and *QRpts4*.
[c] *Pyrenophora teres* isolate
[d] Total number of lines with R or S allele *QRpt6* and *QRpts4*.
*Significant (P <0.05) or **highly significant (P <0.01) using T-test.

In the CDC Bold/McLeod population, the disease reaction of MMAS resistant and susceptible lines was not significantly different from each other at the seedling stage in the Phytotron (Table 23). It is not surprising as both the parents showed intermediate to susceptible reaction at the seedling and adult-plant stage. Therefore, these lines were not tested in the field 2008.

In the SB01045/TR02367 population, MMAS resistant lines showed lower IR than that of susceptible lines at the seedling stage against NFNB isolate WRS858 and SFNB isolate WRS857 and at the adult-plant stage in the field (Table 23). This further confirms that MMAS for net blotch using HVM74 (*QRpt6*) alone or in combination with HVM03 (*QRpt4*) could be used in a practical barley breeding program.

Publications

Papers published in the peer-reviewed journals

1. Grewal, T.S., B.G. Rossnagel, C. Pozniak and G. J. Scoles. 2008. Mapping of quantitative trait loci associated with barley net blotch resistance. *Theor. Appl. Genet.* 116: 529-539.

2. Grewal, T.S., B.G. Rossnagel, and G. J. Scoles. 2008. The utility of molecular markers for barley net blotch resistance across geographic regions. *Crop Sci.* (In Press).

Papers presented at conferences

Oral Presentations

1. Grewal, T.S., B.G. Rossnagel, and G. J. Scoles. 2008. Net blotch quantitative trait loci – their practical application in barley breeding. Paper to be presented at North American Barley Researchers Workshop 2008 at Madison, WI, USA on October 26-29, 2008.

2. Grewal, T.S., B.G. Rossnagel, and G. J. Scoles. 2008. Barley net blotch quantitative trait loci – mapping, validation and their utilization. Paper to be presented at 29th Plant Pathology Society of Alberta Annual Meeting and Third Joint Meeting with Saskatchewan Canadian Phytopathological Society Annual Meeting at Lloydminster on October 20-22, 2008.

3. Grewal, T.S., B.G. Rossnagel, and G. J. Scoles. 2007. Seedling vs adult-plant net blotch resistance in barley. 2007 Saskatchewan Canadian Phytopathological Society Annual Meeting at Saskatoon, SK on Dec. 4, 2007. *Can. J. Plant Pathol.* 30: 385 (Abst.).

4. Grewal, T.S., B.G. Rossnagel, and G. J. Scoles. 2006. Mapping of quantitative trait loci associated with barley net blotch resistance. 2006 Saskatchewan Canadian

5. Grewal, T.S., B.G. Rossnagel, R. G. Stonehouse and G. J. Scoles. 2006. Genetics of resistance to net blotch (*Pyrenophora teres*) in barley, molecular marker development and validation. Pages 33-35 *in* Proceedings of the 3rd International Workshop on Barley Leaf Blights. Edited by T.K. Turkington, D.D. Orr, and K. Xi. July 23-27, University of Alberta Conference Centre, Edmonton, Alberta, Canada.

6. Grewal, T.S., B.G. Rossnagel, and G. J. Scoles. 2005. Molecular markers linked to disease resistance genes - are they applicable world-wide? Net blotch of barley - a case study. Paper presented at 2005 Saskatchewan Canadian Phytopathological Society Annual Meeting at Saskatoon, SK on Dec. 5, 2005. *Can. J. Plant Pathol.* 28: 332 (Abst.).

Poster Presentations

1. Grewal, T.S., B.G. Rossnagel, and G. J. Scoles. 2007. Net blotch of barley – development and application of molecular markers. 5th Canadian Barley Symposium at Winnipeg, MB on June 25-29, 2007.

2. Grewal, T.S., B.G. Rossnagel, C. Pozniak and G. J. Scoles. 2006. QTL mapping of net blotch resistance using DArT. Pages 111-113 *in* Proceedings of the 3rd International Workshop on Barley Leaf Blights. Edited by T.K. Turkington, D.D. Orr, and K. Xi. July 23-27, University of Alberta Conference Centre, Edmonton, Alberta, Canada.

3. Grewal, T.S., B.G. Rossnagel, and G. J. Scoles. 2005. Can we use Australian identified molecular markers for barley net blotch resistance in western Canadian breeding programs? Poster presented at the 18th North American Barley Researchers Workshop and 4th Canadian Barley Symposium at Red Deer, Alberta on July 17 - 20, 2005. pp. 62.

(d) Personnel

Name of Person	Designation	Amount of Time Spent
Dr Tajinder S. Grewal	Professional Research Associate	75%
Ramon Arango	Res. Technical Assistant	October 1, 2007 to Dec. 31, 2007 - 50%
Xiaoxia (Cheryl) Zhao	Res. Technical Assistant	Jan. 14, 2008 – April 30, 2008 – 50%
Yonyou Su	Res. Technical Assistant	May 1, 2008 to May 15, 2008 – 50%
Xiangmin Zhang	Res. Technical Assistant	May 20, 2008 to till date – 50%

(e) Equipment - N/A

(f) Project developed materials – Molecular markers linked to net blotch resistance QTL/genes. These markers are publically available.

(g) Project photos – N/A

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(i) Expense statement - to be submitted by U of S Business Office.